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(54) Title: A METHOD OF ALCOHOLIC FERMENTATION WITH THE CATALYSTS KISSINS AND GAMMA-ALUMINA, AFTER THEIR REGENERATION

(57) Abstract

A method of alcoholic fermentation with the catalytic action of the catalysts Kissiris and γ-alumina, which can find an application in the industry, since these catalysts are regenerated, after the fermentation of a batch, with hot water treatment. Thus, they can be used again for a large number of batches with no productivity decrease. With these catalysts we can ferment completely diluted molasse with initial 17.3^{0}Be and obtain acceptable yields of ethanol to the industry (0.45-0.49 g/g) and productivity (40-60 g/l/24hr) with alcohol content 11 % v/v. Without the use of the catalysts we have a productivity of 7.5-18 g/l/24hr, yields of 0.28-0.39 g/g and an alcohol content of 6-9 % v/v. Analogous results we obtain with γ-alumina in fermentations of the sugars fructose, sucrose, invert sugar e.t.c. The use of γ-alumina powder, 850 μm, gave, with stirring, better results than the pellets.

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DESCRIPTION

A METHOD OF ALCOHOLIC FERMENTATION WITH THE CATALYSTS KISSINS AND GAMMA-ALUMINA, AFTER THEIR REGENERATION

The invention is referred to a method of alcoholic fermentation with two catalysts , kissiris and γ-alumina, a method which can be applied in Industry, since their catalytic action remains constant from one fermentation batch to the next. This is achieved by the regeneration of these catalysts with water treatment after a fermentation batch.

Following the energy crisis of 1973 , there appears a turn towards the production of energy from renewable sources. Such a source is the biomass , from which we may produce bioethanol through the alcoholic fermentation of sugar-rich raw materials. The alcohol may be used as an automobile fuel in a mixture with gasoline (gasohol) or may replace gasoline completely with proper modification of the engine. However , the production of fuel alcohol with the present techology faces the fact that consumption of energy in the production of alcohol is almost equal to the thermal energy produced by its combustion. Also, the production cost of alcohol in relation to that of gasoline is an additional factor wich discourages its use as an automobile fuel.

The alcohol produced worldwide with molasse as a raw material, is used as a starting material for the manufacture of alcoholic beverages (liquors, brandy, ouzo e.t.c.). It is possible, though, a part of molasse and extracts of sugar beets from which the molasse is produced,to be used as a raw material for the production

of fuel alcohol following the reduction of energy consumption and of its production cost is desirable also in the production of potable alcohol which is manufactured in the factories of the existing technology.

The cost reduction can be achieved through the increase of the rate of alcoholic fermentation, while the energy cost can be reduced through the increase of the alcohol content of the fermentation product. The last has an effect on the reduction of the oil consumption in the boilers of the factory, while the rate increase of the fermentation increases the productivity and decreases the construction cost of the fermentors.

γ-Alumina proved to increase significantly the alcoholic fermentation of sucrose, invert sugar and molasse, while increases the final alcohol content of the fermented liquid, in comparison to that in which no γ-alumina is added.

Kissiris or thiraiki gi increases significantly the rate of alcoholic fermentation of molasse. It can be used in a large number of repeated batches of fermentation with no decrease of its catalytic action , only after its regeneration. The regeneration can be done after a fermentation batch by simply washing it with water. The washing is done rapidly with hot water. Depending on the sourse of molasse, kissiris was found to show a decrease of catalytic action after three to nine batches of fermentation. However, if it is to be used in a large number of batches without replacement,something which has a direct effect on the cost, it must be regenerated. This treatment is very important for the production of alcohol from molasse with increased productivity and

decreased energy consumption using this mineral. This is also true for γ-alumina, mainly in the case of molasse.

Thus, it can be stated, that kisiris as well as γ-alumina are two catalysts (or rather strengtheners of enzyme catalytic action) of alcoholic fermentation of molasse and other sugars or sugar-rich raw materials, which in order to be used, should be used in a large numper of batches with the same amount of catalyst. This goal can be achieved, if the catalytic action of the catalyst remains constant from one batch to the next and for a large number of batches. This, however, can be realized through the regeneration of catalyst, the methodology of which is presented in this invention. The two catalysts show an increase of productivity in the fermentation reactors as well as an increase of the alcohol content as compared to the fermentations without Kissiris and γ-alumina or with those with an unregenerated catalyst. The latter can take place also in the industry using free cells with catalyst proposed and constitute the traditional mode of fermentation in industry.

Kissiris

The mineral kissiris is known as elaphropetra or thiraiki gi. It is a porous material and contains chiefly SiO_2 , Al_2O_3 and other inorganic oxides. It is of volcanic origin and occurs in the island Thira (Santorini) and other Greek islands. In our experiments it was used after washing with water.

A. Fermentations of molasse with kissiris in successive batches after regeneration of kissiris.

We placed in a 1-liter beaker 100 - 800g kissiris, 500 ml diluted molasse 10-17.4 ^{0}Be which contains as a nutrient phosphate salt of ammonium or of alkali metals (K, Na) with or without sterilization and baker's yeast (Saccharomyces cerevisiae) or some other yeast srain with a cell concentration greater than 10g/l (pressed yeast).

In order to compare kinetic factors in the presence of kissiris, we ran simultaneous fermentations in the absence of it using the same volume of molasse in the same initial ^{0}Be, the same amount of the same yeast and under the same, generally, conditions. The experiments were carried out at 25-32 ^{0}C with no stirring.

The pH of the molasse to be fermented was adjusted to the optimum range. When the fermentation was over, the liquid product was decanted and the kissiris was washed 3 times with 500ml water of temperature 15-100 ^{0}C or with boiling water under steam pressure, for the regeneration of the catalytic action. The catalyst was then used for the next fermentation batch which was carried out exactly as the previous one and this process was repeated many times.

A part of the results obtained are given in Table I. The method was repeated with powdered kissiris and stirring and the results were similar. The experiment was carried out also in a 3-l feed batch reactor with

pieces of kissiris, a dosometric pump and the same conditions and the results were similar.

TABLE I.

Results of the method with molasse.

Ferment. batch	Initial ^{0}Be density	Final ^{0}Be density	Ferment. time (hr)	Ethanol Conc. (g\l)	Ethanol Productivity (g\l\24hr)	Ethanol Yield Factor (g\g)
1st	17	10.8(6,4)*	160(40)	50(89)	7.5(5.34)	0.28(0.49)
12th	16.6	10.8(8.9)	160(26)	-	-	-
57th	17.2	8.3(6.6)	95(50)	72(89)	18(42.7)	0.39(0.48)
58th	17.3	8.3(6.7)	95(49)	68(83)	17(40.7)	0.37(0.45)

* Values in parentheses are those obtained in the presence of kissiris.

γ-Alumina.

Pellets of γ-alumina Houndry Ho 415 with a porosity 0.45 cm^3 /g and specific surface area 1.40m^2 /g. γ-Alumina was also employed as powder after sifting it through sieves.

B. Fermentations of molasse with γ-alumina pellets in successive batches.

We placed in a 1-liter beaker 70-1000 g of γ-alumina pellets, 500 ml diluted molasse 10-17.4 ^{0}Be which contains as nutrient phosphate salt of ammonium or of alkali metals (K, Na) with or without sterilization and baker's yeast (Saccharomyces cerevisiae) or some other strain of yeast with a cell concentration greater than 10 g/l (pressed yeast).

In order to compare kinetic factors in the presence of γ-alumina we carried out simultaneous fermentantions in the absence of γ-alumina, using the same volume of molasse, in the same initial ^{0}Be and with the same amount of the same yeast and under the same,

generally, conditions. The experiments were performed at 25-32 ^{0}C and no stirring.

The pH of the molasse to be fermented was adjusted to the optimum range. The pH was adjusted also during the fermentation to the optimum range. When the fermentation was completed, the liquid product was decanted and the alumina was washed 3 times with water of temperature 5-100 ^{0}C or with boiling water under steam pressure, for the regeneration of the catalytic action. The catalyst was used then for the next fermentantion batch, carried out exactly as the previous one, and the process was repeated many times.

A part of the results obtained are presented in Table II. The experiment was repeated in a 3-l feed batch reactor employing a dosometric pump and the same conditions and gave similar results.

TABLE II.

Ferm-ent. batch	Ini-tial conc. of sugar (g/l)	Ferm-ent time (hr)	Etha-nol conc. (g/l)	Etha-nol Produ-ctivity (g/l/24hr)	Resi-dual sugar (g/l)	Conver-sion (%)	Ethanol Yield Factor (g/l)
1st	179.5	144(57)	53.9(78.4)	9.0(33.0)	66.9(19.0)	62.7(89.4)	0.30(0.44)
8th	196.0	120(65)	58.4(83.2)	11.7(30.7)	71.0(19.4)	64 (90)	0.30(0.43)
13th	193.0	120(47)	58.4(83.2)	11.7(42.4)	71.0(15.7)	64 (92)	0.30(0.43)
18th	196.5	120(70)	58.4(84.8)	11.7(29.0)	71.0(16.6)	64 (91)	0.30(0.43)

The values in parentheses are those obtained in the presence of γ-alumina.

Since the molasse is a product of sugar beets and sugar canes, the effect should be analogous for their extracts.

C. Fermentations of sucrose, fructose and invert sugar solutions.

In a 1-liter beaker were placed 70-1000 g of γ-alumina pellets, 500 ml of sucrose, fructose or invert sugar solu-tions, 215 g/l containing 1g/l $(NH_4)_2SO_4$, 5 g/l $MgSO_4$ and 4 g/l of yeast extract. To this mixture were added baker's yeast (Sacharomyces cerevisiae) or some other strains of yeast with a cell consentra- tion greater than 10 g/l (pressed yeast). In order to compare the kinetic factors of fermentations in the presence of γ-alumina, fermentations were carried out with the same volume of sugar solution, same concentrations of nutrients, same amount of the same yeast and the same, generally conditions, with no γ-alumina pellets. The experiments were performed at 20-32 ^{0}C with no stir-ring. The pH was adjusted to the optimum range for sucrose and to 3.2-6.5 for invert sugar and fructose. A part of the results obtained appear in Table III.

TABLE III.

Sugar	Initial sugar conc.	Ferment. time	Residual sugar	Ethanol Yield Factor
	(g/l)	(hr)	(g/l)	(g/g)
Fructose	215	24(10) *	29(17)	0.41(0.44)
Sucrose	215	25(16)	52(10)	0.36(0.46)
Invert Sugar	215	24(14)	48(12)	0.37(0.45)

* The values in parentheses are those obtained in the presence of γ-alumina.

<u>D. Repeated fermentations of glucose solutions with γ-alumina pellets, following the regeneration of catalytic action.</u>

In a 1-liter beaker were placed 70-1000 g of γ-alumina pellets and 500 ml of 215 g/l glucose solution containing 1 g/l KH_2PO_4, 1 g/l $(NH_4)_2SO_4$, 5 g/l $MgSO_4$ and 4 g/l of yeast extact.

To the mixture was added baker's yeast (Saccharomyces cerevisiae) or some other strains of yeast in cell concentration greater than 10 g/l (as pressed yeast).

In order to compare the kinetic factors of fermentation in the presence of γ-alumina, fermentations were carried out in the absence of γ-alumina in the same volume of glucose solutions with the same concentrations of nutrients the same amount of the same yeast and the same, generally conditions. The experments were run at 20-32 ^{0}C with no stirring and the pH adjusted to 3.2-6.5.

At the end of the fermentation the liquid was decanted and the pellets were used in another fermentation batch with or without regeneration if this is not required. The regeneration is accomplished with water of temperature 15-100 ^{0}C or with boiling water understeam pressure. Thus, a large number of fermentation batches were carried out with the same amount of γ-alumina.

The experiment was carried out in a 3-l feed batch reactors, employing a dosometric pump and the same conditions and gave similar results.

E. Fermentations of glucose with γ-alumina powder.

In a 1-liter beaker containing 70-300 g of powdered alumina were added 500 ml of 215 g/l glucose solution containing 1 g/l KH_2PO_4 , 1 g/l $(NH_4)_2SO_4$, 5 g/l $MgSO_4$ and 4 g/l of yeast extact.

To the mixture was added baker's yeast (Saccharomyces cerevisiae) or some other strains of yeast in cell concentration greater than 10 g/l (as pressed yeast). During the fermentation the mixture was stirred with a magnetic stirrer. In order to compare the kinetic factors of fermentation in the presence of γ-aluminum powder, fermentations were performed without γ-alumina, int the same volume of glucose solutions, with the same concentrations of nutrients, the same amount of the same ueast and under the same, generally, conditions. The pH was adjusted to 3.2-6.5 and the temperature to 20-32 ^{0}C. The results obtained are shown in Table IV.

TABLE IV.

Particle size of γ-alumina (μm)	Initial sugar conc. (g/l)	Ferment. time (hr)	Residual sugar (g/l)	Ethanol Yield Factor (g/g)
No γ-Al2O3	215	70	20	0.42
pellets	215	32	16	0.43
850	215	24	2	0.48
212	215	29	15	0.43

CLAIMS

"A METHOD OF ALCOHOLIC FERMENTATION WITH THE CATALYTIC ACTION OF THE CATALYSTS KISSIRIS AND γ-ALUMINA, AFTER THEIR REGENERATION"

1. A method of alcoholic fermentation with the use of kissiris and γ-alumina, characterized by the fact that the fermentation of molasse, sucrose, invert sugar, glucose, juices or extracts of raw materials can be accomplished, in the presence of each one of these materials separately.

2. A method of alcoholic fermentation, as stated in the claim 1, characterized by the fact that the two catalysts can be used in successive batches, after their regeneration with water of temperature 15-100 ^{0}C, a process enabling them to be used for a large number of batches with no reduction of their productivity.

3. A method of alcoholic fermentation, as stated in the claim 1,characterized by the fact that the two catalysts can be used in successive batches, after their regeneration with boiling water under steam pressure, a process enabling them to be used in successive batches with no reduction of their productivity.

4. A method of alcoholic fermentation, as stated in the claim1, characterized by the fact that the γ-alumina in the fermentation of molasse can be used also in the powder form, with results which are better than those obtained with pellets of γ-alumina.

5. A method of alcoholic fermentation, in accordance with the claims 1, 2, 3 and 4 characterized by the fact

that the fermentation with glucose can be accomplished with the stirring of γ-alumina powder.

6. A method of alcoholic fermentation, in accordance with the claims 1, 2 and 3, characterized by the fact that the kissiris in the molasse fermentation can be used in powder form.

7. A method of alcoholic fermentation, in accordance with the claims 1, 2, 3, 4, 5 and 6 characterized by the fact that the fermentation can be accomplished with any part of yeast which affects an alcoholic fermentation.

8. A method of alcoholic fermentation, in the molasse and glucose fermentations in successive batches, 4, 5, 6 and 7 in accordance with the claims 1, 2, 3, characterized by the fact that the fermentation can be accomplished in a feed batch reactor.

9. A method of alcoholic fermentation, in accordance with the claims 1, 2, 3, 4, 5, 6, 7 and 8 characterized by the fact that there can be any relationship of weight of γ-alumina or kissiris to the volume of the liquid which results from the description.

10. A method of alcoholic fermentation of molasse, as it is stated in the claims 1, 2, 3, 4, 5, 6, 7, 8, 9, characterized by the fact that it can be fermented in 10-17.4 ^{0}Be and contents one of the phospate salts of ammonium or one of the corresponding to the alkali metals (K, Na).

INTERNATIONAL SEARCH REPORT

International Application No PCT/GR 92/00008

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12P7/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category°	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	JOURNAL OF FERMENTATION AND BIOENGINEERING vol. 69, no. 2, 1990, SUITA, JAPAN pages 93 - 97; TSOUTSAS, T. ET AL.: 'Kissiris: a mineral support for the promotion of ethanol fermentation by Saccharomyces cerevisiae.' see the whole document ---	1,7-13
X	BIOMASS vol. 21, no. 3, 1990, ENGLAND pages 189 - 206; HAMDY, M.K. ET AL.: 'Continuous ethanol production by yeast immobilized on to channeled alumina beads.' see page 189, paragraph 1 - page 195, paragraph 1	1,7-11, 13
Y	see page 199, paragraph 2 ---	2-3
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° Special categories of cited documents :[10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
03 SEPTEMBER 1992	09.09.92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	BEVAN S.R. SBevan

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	WORLD PATENTS INDEX Section Ch, Week 7604, Derwent Publications Ltd., London, GB; Class D, AN 76-06842X & SU,A,429 088 (PSALOM P G) 20 June 1975 see abstract ---	2-3
X	BIOTECHNOLOGY & BIOENGINEERING vol. 34, no. 1, 5 June 1989, NEW YORK pages 121 - 125; KANELLAKI, M. ET AL.: 'Ethanol production by Saccharomyces cerevisiae promoted by gamma-alumina.' see the whole document ---	1,8-9, 11-13
P,X	APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY. vol. 31, no. 1, October 1991, CLIFTON, N.J. pages 83 - 96; ICONOMOU, L. ET AL.: 'The promotion of molasse alcoholic fermention using Saccharomyces cerevisiae in the presence of gamma-alumina.' see the whole document ---	1-5,7-13
P,X	APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY. vol. 30, no. 2, August 1991, CLIFTON, N.J. pages 203 - 216; KOUTINAS, A.A. ET AL.: 'Continuous potable alcohol production by immobilised Saccharomyces cerevisiae on mineral kissiris.' see the whole document ---	1,6-9, 11-13